CHAPTER 31

APPLICATIONS OF NANOTECHNOLOGY IN FISH HEALTH

Fariha Latif*[1], Sana Aziz[2], Safina Kousar[3], Rehana Iqbal[1] and Muhammad Mudassar Shahzad[4]

[1]Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan
[2]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
[3]Department of Zoology, Government College for Women University, Faisalabad, Pakistan
[4]Department of Zoology, University of Education, Lahore, Pakistan
*Corresponding author: farihalatif@bzu.edu.pk

INTRODUCTION

Nanotechnology

Nanotechnology is a highly favorable field that extends to many zones of scientific and technological applications. Nanoparticles (NPs) are defined as particles that have a dimension in the range between 1–100 nm (Nowack and Bucheli 2007), that are one hundred to thousand times smaller in diameter than the paper thickness or human hair. A remarkable rise in applications of NPs have been indicated by many researchers over the last decades due to their unique properties (specifically particle surface area, size, surface charge, reactivity and shape) relative to their dissolved counterparts or bulk forms (Maurer-Jones et al. 2013) Additionally, their unique features have raised concerns due to their physiological responses to biological systems after quick interactions with surrounding materials. The emergence of NPs into aquatic ecosystems related to overuse has led investigators to study their various characteristics, behavior, sources, and ecotoxicological effects (Bundschuh et al. 2018). Although, many studies have informed the impacts of nano-based materials on human health, their ecological impacts, comprising fate, mechanism of toxicity, have been measured in recent years to encourage the sustainable use of these nanomaterials.

Main Sources of Nanoparticles

Nanoparticles can enter the surrounding environment from different natural and anthropogenic activities. Natural sources include soil erosion, forest fires, dust storms, and volcanic activities and engineered nanoparticles from anthropogenic sources can enter the ecosystem during their life cycle through three main emission strategies,

(i) release during manufacturing of nano-enabled materials
(ii) release during their excessive use and
(iii) release after direct disposal of products having NPs (waste handling).

They can be entered either directly into the environment or indirectly through a mechanical system such as landfills or wastewater treatment plants.

Bioavailability and Transformational Processes of Nanoparticles

Aquatic media can change the fate and performance of toxicants and their subsequent e bioavailability to fish, is well recognized. For example, metal speciation models reported that water chemistry such as calcium contents, pH, and hardness affect the concentration of metallic ions and their bioavailability to aquatic animals (Leeuwen and Town 2005). Abiotic factors (e.g., pH, temperature and water hardness), occurrence of natural organic matter (NOM) and characteristics of colloid chemistry eventually regulate the fate and behavior of synthetic NPs in water and this phenomenon has been studied (Klaine et al. 2008) and various significant ideas have arisen which are closely related to fish ecology: (1) NPs have ability to build suspensions in fluids, they don't take solution forms regarding the traditional water chemistry; (2) NPs take the form of agglomerates (weak interaction between particles), or aggregates (e.g. combinations of particles) and this colloidal nature strongly influence their bioavailability; (3) colloidal nature is greatly affected by few abiotic parameters of aquatic environment, and the utmost significant fact here are water pH, existence of divalent ions; though temperature of water and occurrence of natural organic substances in aquatic environment may also be vital.

Nanoparticles have undergone various modifications depending on them inherit properties and characteristics of aquatic media. Biological, chemical, and physical alterations are the key procedures that clearly explain the activities of NPs in aquatic environments (Lowry et al. 2012). Physical practices encompass different agglomeration, accumulation, and deposition. Chemical practices cover photochemical reactions, suspension, and redox reactions like oxidation, while biological applications include the bacterial breakdown and bacteriological modifications (Lead et al. 2018).

Chemical Transformation

Redox reactions include the transmission of electrons between chemical entities in natural ecosystems. Metals such as iron and silver repeatedly experience redox processes (Liu et al. 2005). Three kinds of ecosystems can be described on the basis of redox-state: oxidizing ecosystem having rich oxygen level like

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aerated soils and natural aquatic water, reductive ecosystem that face depletion of oxygen e.g., water of ground and rocks rich in carbon and dynamic redox ecosystem such as tidal zone in which various oxido-reduction reaction can occur. Photo-oxidation and photo-reduction are the reactions that are catalyzed by sunlight and change the oxidation state of nanoparticles and the tenacity of reactive oxygen species. For example, TiO2 and CNTs are activated by light and are capable of producing ROS (Chen and Jafvert 2011). Activities and performance of nanoparticles can change by large molecules and when organic and inorganic ligands are adsorbed on nanoparticles.

Physical transformations

Physical transformations occur at all phases of the life history of nanoparticles, and it take the form of aggregation and agglomeration. Aggregation and agglomeration are the same processes and can be transformed into each other. Aggregation is defined as close bonding between particles via electrostatic forces of attraction that may result in a reduction in surface area. Two types of aggregation are homoaggregation that occurs between the same type of NPs and heteroaggregation that occurs between NPs and other materials of the environment. Agglomeration increases the size of nanoparticles that affect their fate, behavior, and toxicity. Less surface area of nanoparticles decreases toxicity, which affects in turn ROS generation or suspension (Lowry et al. 2012). Spongy aggregates are capable of forming sediments than the compact form which remains suspended in aquatic media and undergoes corrosion processes producing smaller particles that absorb natural organic matter (Chekli et al. 2015).

Biological Transformations

Biological transformation is the third type of transformation that particularly affects nanoparticles. In aquatic environments where nanoparticles are directly associated with living creature's biological transformation is unavoidable. The exposure of nanoparticles to aquatic organisms occurs via many ways such as redox reactions, alterations in nanoparticles surface by coating or contact with other materials. These alterations will affect the reactivity, behavior, and surface chemistry of NPs. It has been proven that occurrence of redox responses in bacterial species (Shewanella and Geobacter) may lead to Ag+ reduction and the formation of silver nanoparticles. Moreover, it was described that transformation of PEG (polyethylene glycol) coverings on nanoparticles indicates their aggregation (Kirschling et al. 2011). Finally, the fate and performance of nanoparticles are different in different aquatic environments. The behavior of suspended organic matter and colloidal particles in sea water would be entirely different due to estuarine systems (Guzman et al. 2006). It was found that ENPs transformational processes directly correlate with ecological conditions of aquatic media (Ju-Nam and Lead 2008).

Applications of Nanotechnology on Fish Health

Fisheries and aquaculture sectors can be developed with the help of modern nanotechnology like quick disease recognition, increasing the fish power to absorb chemicals, hormones and injections quickly. Present prediction from National Science Foundation (USA), computes the worth of the global nanotechnology industry at USD one trillion by 2015. This could be possible due to extensive use of nanomaterials, not only in electrical and materials sectors but also in domestic, agriculture divisions involving fish culture and its services in biological sciences for detection of biomolecules, treatment of cancer, expansion of non-viral vectors for genetic engineering, transmission of foreign DNA, targeting medicine supply, clinical surgeries etc. although more knowledge is needed to increase probable use of nanotechnology in fish culture. Fish is considered main constituent for diet of poor people, basically relies on indispensable food and fish meat provide too much protein content. Almost 150 g of protein from fish meat provides 50-60% of an individual's daily requirement (Mohanty 2015). Solid confirmation data highlights the fact that intake of fish meat, especially fatty fish, decreases the chance of coronary heart disease (CHD) deaths. Although, fish culture is still under insecurity in terms of tapping interrogation on its sustainability, where large number of pollutants from aquaculture exert harmful effects on their productivity and the aquatic ecosystems. In this context, nanotechnology is emerging as an innovative field for skill and technology for agriculture, development and revolution (Rodrigues et al. 2017). Table 1 shows the applications of different nanoparticles in fish health. Nanotechnology in fish has various direct and indirect applications:

Direct Application

i. Fish growth
ii. Fish feed
iii. Reproduction and gonadal maturation
iv. Fish disease management

Nanoparticles play a central role in the effective supply of nutrients, vitamins and trace minerals as nano-feed additives e.g., selenium, iron and vitamin C (Jimenez-Fernandez et al. 2014). Fish feed comprising nanosized nutrients help in their assimilation and passage in the intestine, to increase their development, reproduction and immunity of fish (Chris et al. 2018). The services of nanotechnology in controlling fish illnesses are more effective as compared to chemicals that exert numerous harmful effects like aquatic pollution, resistant bacterial strains and deposition of chemical remains.

Nanomaterials are known as antiviral, antibacterial, anti-parasitic and antifungal. Furthermore, poly-lactic-glycolic acid and chitosan NPs play a significant role in medicine and hormone supply, and immunization (Bhat et al. 2019). Definitely, nano-vaccination has many benefits over traditional methods, as it increases constancy, bioavailability, and residual period (Kitiyodom et al. 2019). Moreover, the utmost prominent service of nanotechnology in fisheries is its use in quick and real identification of fish pathogens (Elsheshtawy et al. 2019).

i. Fish Growth

Proper growth rate of desired species defines the profitability of the aquaculture industry. Aquaculturists have always desired for a new element that can promote growth of the cultured fish species. Nanotechnology has extensive potential for enhancement of fish as immunomodulators (Kumar et al. 2019) and growth promoters (Zhou et al. 2009) and when provided with fish diet as supplement. Researchers from the Russian

Table 1: Applications of frequently used nanoparticles in fish health

NPs	Test Organism	Target Organs/systems	Results	References
Chitosan	*Danio rerio*	Biomarkers and whole organism	overexpression of HSP70, opaque yolk, decreased hatching, increased mortality	Hu et al. (2011)
Cerium Oxide (CeO_2)	*Paracentrotus. lividus*	Survival, bioaccumulation, nervous system, gene expression	Ce bioaccumulation in digestive, the reproductive and immune systems	Falugi et al. (2012)
Silicon Oxide (SiO_2)	*Danio rerio*	Survival, development, behavior	Decreased hatching rate of embryos, increased mortality, decreased total swimming distance	Duan et al. (2013)
Gold (Au)	*Hediste diversicolor*	Behavior, oxidative stress	Impaired borrowing behavior and feeding, significantly increased stress-related biomarkers	Mouneyrac et al. (2014)
Silver (Ag)	*Synanceia verrucosa*	Breathing behavior, reproduction, ROS, antioxidant enzymes	Decrease breathing, increased production of ROS and antioxidant enzymes	Volker et al. (2015)
Selenium (Se)	*Tor putitora*	Digestive System	Positive effects on the physiological aspects	Khan et al. (2016)
Copper Oxide (CuO)	*Daphnia magna*	Growth, reproduction, Genotoxic effects in hemocytes	Low impact on the growth and reproduction	Adam et al. (2015)
Iron (Fe)	*Oryzias latipes*	Breathing system, oxidative stress	Exposure to NPs led to a combination of hypoxia and production of ROS	Chen et al. (2013)
Aluminum Oxide (Al_2O_3)	*Bacterial activity*	Antibacterial activity of Al-NPs	No inhibition exhibited by Al NPs against different bacterial isolates	Swain et al. (2014)
Tin Oxide (SnO_2)	*Paracentrotus lividus*	Survival, bioaccumulation, nervous system, gene expression	Tin bioaccumulation in digestive, reproductive, and immune systems,	Falugi et al. (2012)
Lanthanum (La)	*Daphnia magna*	Survival and motility	Immobilization of *D. magna*	Balusamy et al. (2015)
Titanium Oxide (TiO_2)	*Hediste diversicolor*	Oxidative stress	Lipid peroxidation and nitric oxide production increased, GSH activity decreased	Zhu et al. (2011)
Zinc Oxide (ZnO)	*Crassostrea gigas*	Zn bioaccumulation, pathological destruction, oxidative stress	Induction of oxidative stress, zinc bioaccumulation in gill and digestive glands	Trevisan et al. (2014)
Zinc Oxide (ZnO)	*Labeo rohita*	Oxidative stress	Lipid peroxidation, Superoxide dismutase and catalase decreased	Aziz et al. (2020)

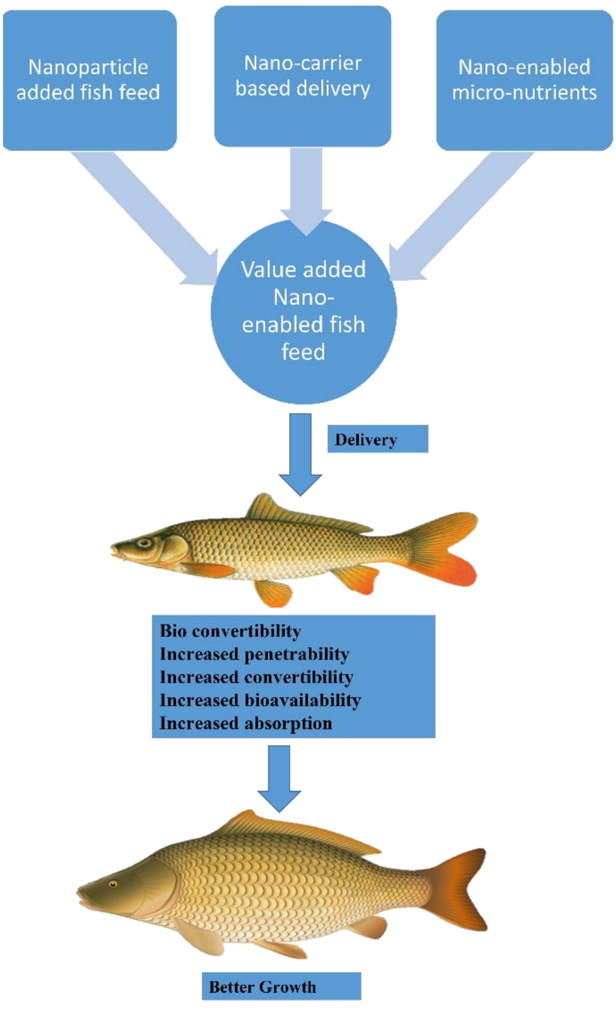

Fig. 1: Applications of nanomaterials in fish feed.

Academy of Sciences have described that both sturgeons and young carp displayed a higher growth rate (24% and 30% respectively) after exposure to an iron nanoparticles-based diet. Studies have reported that nano-selenium as diet supplement could improve the weight, muscle Se concentration and antioxidant response of crucian carp (Carassius auratus gibelio) than other selenium- based sources (Ashouri et al. 2015). It can be indicated that fish health conditions can be influenced by nano-techniques in fisheries and aquaculture, and pond-ecosystems have shown great potency for nanomaterials. Various nanotechnological processes can provide high quality preserved food. Silver NPs have been reported to enhance growth, and metalloprotease level in zebrafish (*D. rerio*). Similarly, increased growth patterns were found in common carp (*C. carpio*) and grass carp (*C. idella*) and after exposure to selenium and zinc oxide nanoparticle-supplemented diets, respectively (Saffari et al. 2017). Magnesium oxide NPs are found to stimulate development of *M. rosenbergii* (Srinivasan et al. 2016). Moreover, Cu-NPs have been reported to promote growth and immunity of sea beams (El-Basuini et al. 2016). *Ctenopharayngodon idella* (grass carp) and *Labeo rohita* fed with ZnO nanoparticle supplemented diets lead to higher rates of RBC and growth.

ii. Fish Feed

One of the most significant applications of nano-techniques in aquaculture and fish health is in feed preparation, where the NPs are effective directly for growth enhancement, nutrient delivery, and feed production per unit time. Apart from these three major areas, efforts are being made in the use of NMs to change the texture, structure, and quality of feed, as well as the preparation, processing, packaging, storage, transportation, and traceability of fish feed, which are also the major fields where nanotechnology is playing its role (Figure 1).

Chitosan NPs are gaining popularity in the animal feed industry because they are a polysaccharide "poly (1,4--D-glucopyranosamine)" with low toxicity, immunogenicity and antimicrobial potential (Vendramini et al. 2016). Different studies have shown the usage of chitosan NPs to increase the shelf life and availability of nutrients in fish. Nanoparticles have the ability to penetrate the intestinal fish epithelium and chitosan NPs have shown promising results in delivering ascorbic acid to the targeted organs of the fish when fed with NP-infused feed due to their reduced size (Jimenez-Fernandez et al. 2014).

Undoubtedly nutraceuticals are recognized to show an important role in immunological parameters and growth. however, their proper incorporations needed higher costs. That's why, deep care should be followed during their usage to avoid wastage and maximize their utilization. In fisheries, literature is available that supports nanotechnological techniques in the actual distribution of dietary nano-supplements and nutraceuticals. These systems are basically designed to increase the bioavailability, nutrients efficacy by enhancing their solubility and defense from extreme conditions of the gut. Addition of 1 mg of Selenium nanoparticles in per kg diet displayed important role in (*Cyprinus carpio*) growth and antioxidative system of common carp than control ones (Ashouri et al. 2015). Moreover, selenium (Se), manganese (Mn) and zinc (Zn) NP as a supplement in diets enhanced stress resistance and bones strength of gilthead seabream (Sparus aurata) (Izquierdo et al. 2017).

Diets having iron NPs as a supplement and and probiotic (*Lactobacillus casei*) significantly increased growth in rainbow trout (Mohammadi et al. 2015), whereas diets having MnO-NPs significantly improved growth and immunity of prawn (*M. rosenbergii*) (Asaikkutti et al. 2016). Likewise, copper NPs as supplement at 20 mg/kg significantly improved growth rate, specific and non-specific immunity of prawn, (*M. rosenbergii*) and red sea bream, (*Pagrus major*) (El Basuini et al. 2017). Gold nanoparticles significantly improved the metabolic enzymes, oxidative stress, and hepatotoxic markers, etc. Sharif Rohani and coworkers reported the beneficial effects of Aloe vera NPs-based diets on growth, body composition and survival rate of Siberian sturgeon (*Acipenser baerii*) than control ones. (Sharif et al. 2017). Common carp (*Cyprinus carpio*) fed with ginger nanoparticles per kg feed exhibited 100% relative percentage survival (Korni and Khalil 2017).

Neem (*Azadirachta indica*) built AgNPs showed improved immunomodulation and antibacterial activity in fingerlings of *Cirrhinus mrigala* confronted with *Aeromonas hydrophila* (Rather et al. 2017). Most recently Erdem and co-researchers prepared AgNPs from *Aeromonas sobria* to check their antibacterial properties against various fish pathogens (*E. hermannii, P. rettgeri, H. alvei, M. morganii subsp. Sibonii, C. braakii, A. hydrophila, E. coli* and *E. cloacae*). And for the management of fish health, NPs were approved as antimicrobial agents against *A. hydrophila* (Erdem et al. 2018). Nanoencapsulation technique reveals new assets like the adding of carbon nanotubes into trout fish diet causing in the preparation of hard pelleted feed.

iii. Reproduction and Gonadal Maturation

Fish breeding and reproduction is a vital segment in the fish industry as the brood stock management is a preliminary step in controlled breeding and to get a healthy broodstock, proper gonadal maturation is crucial. This gonadal maturation has been attained by injecting multiple hormones or by adding various supplements to feed during the prespawning phase. Both of these conventional ways have their issues, as injections cause handling stress and occupational pains while the hormonal therapy of testosterone and progesterone through feed supplements has a major drawback of hormones leaching in water during delivery. With the advancement of nanotechnology, this issue has been addressed by the implantation of hormonal pellets by nanocarriers at the prespawning stage under the fish skin (Kailasam et al. 2006). This technique would solve the issue of leaching by increasing the retention period of hormones in the fish body and triggering the maturation of the gonads by gradual and slow release of hormones as the results showed the higher retention time and longer life span of luteinizing hormone in the fish circulation. NPs of chitosan can be used to transfer endogenous hormones and release them in a precise way.

The use of chitosan nanoconjugates with reproductive hormones exhibited promising results as Rather et al. (2013) used chitosan-gold nanoconjugates with LHRH in the female *Cyprinus carpio* to solve the issue of the short life span of LHRH in fish blood. Their results showed that the concentration of reproductive hormone in blood circulation was consistent for a longer period in fish that solved the issue of multiple hormone injections and thus by increasing the relative number of mature eggs and significantly higher fertilization rate in the female fish. The application of nanoconjugates of chitosan and hormones also showed a significant increase in the expression levels of SoX9 (SRY-Box Transcription Factor 9) gene that is associated with the gonadal development of male and female *Clarias batrachus* (Bhat et al. 2016). The plant extract (Eurycomanone) used as nanoformulation of chitosan conjugate injected in the male *Claria smagur* showed a significant increase in the intracellular selenium and calcium level, the improved cellular structure of testes, gonado-somatic index (GSI), and overall better reproductive capacity of the fish (Bhat et al. 2019). The conventional way of injecting brood with the hormones causes another major issue that is the accumulation of hormone residues in the tissues after breeding. This problem was also solved by the minimal accumulating effect of nano-delivery of hormones thus by preventing the retardation of broodstock by hormonal residues that led to the cheaper sale of post-breeding broodstock.

In commercial aquaculture, another common problem is incomplete vitellogenesis in female fishes that leads to malfunctioning of oocyte maturation and ovulation. This issue can be solved by controlling the reproductive process of fish as the production of monosex Tilapia was a breakthrough to avoid premature spawning, small-sized, unmarketable fish, and pond overcrowding. The poly lactic-co-glycolic acid nanoparticles were loaded with fadrozole (an inhibitor of estrogen synthesis), incorporated in the feed of *Oreochromis niloticus*, fed through a nano-drug delivery system. These fadrozole loaded nano-carriers inhibited the production of estrogen hormone in the fish, thereby, producing 100% masculinization of tilapia that attained their marketable size without premature spawning (Joshi et al. 2019).

Another problem that occurs frequently in commercial aquaculture is incomplete vitellogenesis in female fishes, which results in the failure of oocyte maturation and ovulation in the female fishes. It is possible to solve this problem by controlling the reproductive process of fish, as demonstrated by the production of monosex tilapia, which was a breakthrough in the effort to avoid premature spawning, small-sized, unmarketable fish, and pond overcrowding.

In fish culture, before focusing on breeding techniques, the health of the broodstock is more important. For this purpose, the feed enriched with all the necessary microminerals, and vitamins is fed to develop high fecundity in the broodstock. Among these minerals, selenium has shown successful results in improving the breeding, fertilization, and reproductive capacity of male fishes (Sarkar et al. 2015). Similarly, calcium plays a significant role in egg activation, whereas low levels of phosphorous reduce fecundity in female fishes. Vitellogenesis in the fish system can also be improved by the minerals and vitamins delivery because they play a significant role in fish breeding as Vitamin E improves the egg quality, ovulation rates, and gonado-somatic index, while vitamin C influence the vitellogenesis and production of the steroids in fish. Significant work on the nano-carrier of minerals and vitamins in the fish breeding program has shown results; for example, silver nanoparticles in the zebrafish diet triggered the hatching and breeding in zebrafish along with the overexpression of Oct4 protein (Sarkar et al. 2018).

iv. Fish Disease Management

With the advancements in aquaculture technology, various detrimental diseases to organisms are also emerging. A huge annual loss is happening in the fisheries industry due to the continuously increasing incidence of diseases caused by different pathogens. To control this loss, efficient detection methods and preventive measures from pathogenic infestation are considered key elements for enhancing overall productivity and healthy fish products. Different strategies have been developed viz. vaccines, immunostimulants, antibiotics, etc. to deal with the various pathogenic diseases encountered in fish culture. Nanotechnology can significantly contribute to these fields through various innovative methods, also restricting the use of conventional technology in medicine. The following schematic diagram is describing the applications of nanotechnology in fish disease management (Figure 2).

A. Diagnosis

Aquaculture scientists in the recent times are looking for a simple and effective technique for timely detection and diagnosis of main fish pathogens. Nanoparticles have been used to diagnose diseases in a quick and sensitive manner, and these detection methods are known as nanodiagnostics. A highly sensitive immunodiagnostic assay has been formed by loading gold NPs with alkaline phosphatase and antibodies for the treatment of viral white spot syndrome in shrimps (Thiruppathiraja et al. 2011). A similar approach using DNA-loaded AuNPs and LAMP was developed to detect viral white spot syndrome in fish. This method was sensitive, specific, and field-detectable. A similar approach using DNA-loaded AuNPs and LAMP was developed to detect viral white spot syndrome in fish. This method was specific, sensitive, and field-detectable. Yellow head virus in shrimps was visually detected at early stages by combining AuNPs colorimetric assay with LAMP.

Nanosensors are also effective and simple tools for detecting pathogens. Important fish viruses such as betanoda, aquabirna, and salmonid alpha can be detected using a variety of nanosensors (Crane and Hyatt 2011). In fish tissues, bacterial diseases are more common than fungal and viral diseases, and

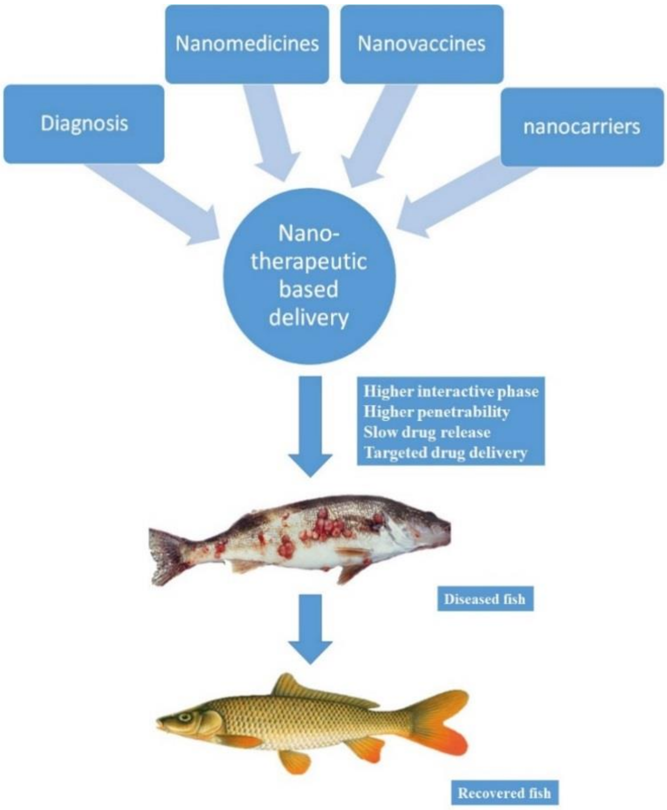

Fig. 2: Nanotechnology in fish disease management.

their early detection is crucial for disease management. Gold nanoparticles have been used in the form of *A. salmonicida* antibody-gold NPs conjugated for the diagnosis of furunculosis in fish (Saleh et al. 2011). Graphene oxide is currently being used in the development of electro-chemical biosensors due to its biocompatibility and specific chemical composition (Natarajan et al. 2017).

These immunosensors also demonstrated a modest, delicate, real-time, and speedy detection method for WSSV in shrimp samples. Biosensing is a modern, unique, and, alternatively, a better quantitative and qualitative method as compared to PCR amplification detection methods. Another electrochemical DNA biosensor was made by the conjugation of Au-NPs and a DNA reporter probe for the detection of fungi (*A. invadans*) in the fish at a lower rate than PCR (Kuan et al. 2013). In an immunomagnetic assay for NNV in grouper fish, Yang et al. (2012) used magnetic NPs covered with anti-NNV antibody from rabbits. A magnetic field applied to magnetic nanoparticles reduced their motility if they were bound to viral antigens, allowing for immunodiagnosis. The titer virus was determined by magnetic immuno-assay.

Unmodified Au-NPs were used to develop a colorimetric assay for detecting of carp virus. Gold nanoparticles were added after the probe, which was complementary for carp virus. If there was any target viral RNA existed, it directly hybridized with the probe, preventing the probe from the gold NPs. The Au-NPs could clump together, causing the solution to change red color into blue. Without the need for early amplification of viral DNA and RNAs, this technique was specific and fast. The same approach was used to develop a specific, sensitive and rapid assay for detecting the virus of DNA, cyprinid herpes virus-3 (Saleh and El-Matbouli 2015).

B. Nanomedicine

Nanomedicine is an emerging field of nanotechnology that offers a plethora of opportunities to improve fish health by employing the intrinsic properties of many types of nanoparticles. Nanomaterials with antimicrobial and therapeutic capabilities, such as nanosilver and zinc oxide, have previously been used to lower pathogens in aquaculture systems. The phenomenon of nanomedicinal is generalized, non-specific, and extensively applicable in animal health.

Researchers are testing nanoparticles' antibacterial properties, which could be valuable nano-medicines for fish species (Table 2). Graphene emerged as viable, low-cost commercial nanomaterial. The oxidized graphene is easily processed and soluble in water (Brisebois and Siaj 2019). Graphene oxide inhibited important pathogens of water such as *P. aeruginosa, S. aureus, E. coli*, and *V. harveyi*. When graphene oxide interacts with pathogens, it induces cell membrane impairment, lysis and mechanical enfolding (Kumar et al. 2019).

Application of metal oxide nanoparticle in aquaculture disease management has been extensively studied and found that nanoparticles such as ZnO-NPs, CuO-NPs, Au-NPs, Ag-NPs, and TiO2-NPs possess the principle of fighting against various disease-causing pathogens (Cheng et al. 2009). ZnO nanoparticles are reported to inhibit the growth of various pathogenic bacteria viz. *Staphylococcus aureus, Flavobacterium branchiophilum, Aeromonus hydrophila, Vibrio species, Pseudomonas aeruginosa, Edward seillatarda Bacillus cereus* and *Citrobacter spp.* (Swain et al. 2014), whereas CuO nanoparticles are effective against Saprolegnia sp. from the host white fish and also used as strong antifungal agent. Au-NPs have been tested against bacterial pathogens of fish and observed to be possess antibacterial properties. Silver nanoparticles are used as an effective antibacterial agent as it releases Ag+ ions which bind to bacterial cell membrane proteins and disrupt the membrane of the bacterial cells. Also, Ag-NPs are found to be effective against multidrug resistant bacteria among them methicillin resistant *Staphyloccus aureus* is one.

Various herbal and phyto-extracts are used to treat fish diseases as potential drugs. Different nanoparticles are prepared at optimized hydrodynamic conditions by using medicinal plant/herbal extracts, and a complex of the phyto-nanoformulation is then administered as a medicine with synergistic effects of both. Phyto-nanoformulation of plant extract and Ag-NPs composite has been observed to be work as antibacterial against *Aeromonas hydrophila*, causing Motile *Aeromonas Septicemia* in fish.

C. Nanovaccines

Disease outbreaks are one of the most significant issues to aquaculture's development and long-term viability. Among various approaches used to solve this major problem, vaccination is one that showed promising results in its utilization in both conventional and modern medicine. In this context, the use of nanoparticle carriers of vaccine antigens such as chitosan and poly-lactide-co-glycolide acid in combination with mild inflammatory inducers may deliver fish. with higher protection not only against bacterial pathogens, but also against certain viral diseases with vaccine-induced side effects.

Nano-delivery of drugs are attributed to some unique properties as controlled particle size, structure, shape, distribution, surface charge, sustained release, regulation, target-specific, multi-route delivery pathways, and degradation of nano-carriers. Nanoparticles are vastly used as drug carriers for multiple reasons including improved bioavailability, prolonged residence time and stability in stomach, highly efficient absorption, dispersion of vaccine antigens to gut-lymphoid cells, and controlled degradation. Nanoparticles as a drug delivery vehicle Chitosan and PLGA NPs have been examined for their effectiveness as a vehicle for delivery of drugs in fish.

Furthermore, nanocapsules can be used to vaccinate fish, which will be difficult to digest and degrade thus showing long-term effects. These nanocapsules contain short strand DNA, that is readily absorbed into fish cells when employed in living medium. The ultrasound method is utilized to burst the capsules, releasing DNA and inducing an immunological response in the fish as a result of the vaccine. This will reduce the cost and effort of disease management, drug and vaccine delivery, and other aspects of aquaculture while lowering the cost of feeding (Assefa and Abunna 2018).

Indirect Applications of Nanotechnology

Indirect applications of nanotechnological techniques are mainly concerned with quality of water:

i. Wastewater treatment
ii. Biofouling control
iii. Removal of heavy metals

Several carbon nanotubes, metal oxide nanomaterials, and natural nano-adsorbents have been used to remove contaminants from the water. Also, water purification from

Table 2: Applications of nanotechnology in fish disease management.

Nanoparticle	Test Organism	Pathogen	Nanomedicines / Nano-vaccines	References
PLGA	*Salmo salar*	IPNV	PLGA Nanoparticle-TA	Munang'andu et al. (2012)
Chitosan	*Danio rerio*	VHSV	NPrgpG, pICrgpG	Kavaliauskis et al. (2016)
PLGA	*Labeo rohita*	*Aeromonas hydrophila*	Np-rOmpW	Dubey et al. (2016)
Alginate	*Oncorhynchus mykiss*	*Ichthyophthirius multifiliis*	OCMCS-HA/aerA-NPs	Heidarieh et al. (2015)
Chitosan	*Saltator maximus*	TRBIV	pDNA-CS-NPs	Zheng et al. (2016)
Chitosan/TPP	*Lates calcarifer*	Nodavirus	pFNCPE42-CS/TPP	Vimal et al. (2014)
Calcium phosphate	*Labeo rohita*	*Aeromonas hydrophila*	SP-CaNP	Behera and Swain (2011)
PMMMA-PLGA	*Oreochromis niloticus*	*Streptococcus agalactiae*	PTRBL/Trx-SIP	Zhang et al. (2015)
PLGA	*Panaeolus olivaceus*	LCDV	pEGFP-N2-MCP	Tian and Yu (2011)
Chitosan	*Acanthopagrus schlegelii*	*Vibrio parahaemolyticus*	pEGFP-N2-OMPK	Li et al. (2013)
PLGA	*Oncorhynchus mykiss*	IHNV	PLGA-pCDNA-G 11 PLGA-pCDNA-G 22	Adomako et al. (2012)
Liposome	*Epinephelus bruneus*	*Vibrio harveyi*	Liposome-V. harveyi	Harikrishnan et al. (2012)
Carbon nanotubes	*Ctenopharyngodon idella*	GCRV	SWCNTs- pcDNA	Zhu et al. (2015)
OCMCS-hyaluronic acid	*Cyprinus carpio*	*Aeromonas hydrophila*	OCMCS/aerA-NPs	Liu et al. (2016)

variety of pathogens shows another preferred outcome of nano-techniques to limit contagious diseases (Tayel et al. 2019). Additionally, the anti-fouling impact of some nano-based materials, by reducing phosphate levels and lessening the growth of micro-organisms and algae, is an important nano technological application, to attain water quality in ponds of fish.

i. Wastewater Treatment

The accretion of food remnants and the presence of a large number of organisms is one of the known issues in fish culture. These pathogens cause many fish diseases by such as bacteria, viruses, fungus, and protozoa. Anti-pathogen drugs have significant risks on pathogen resistance, fish health, non-targeted animals, and the environment. Nanotechnology in water disinfection and sterilization may be a possible answer to all these issues (Tayel et al. 2019). The sterilization efficiency depends on the pollutant type, concentration, nano-material concentration, temperature, and light intensity as well. Nanoparticles of TiO2 were observed to sterilize 98% of E. coli, *V. anguillarum* and *A. hydrophilain* water during sunlight, whereas composite of nanomontmorillonite and Cu showed bactericidal properties to clean fishpond waters (Rati et al. 2014).

The composite of nano-Ag coated zeolite and activated carbon has significantly reduced the Saprolegnia infection in the eggs of rainbow trout. In fish farming, the water quality was also reported to get suitable after the application of chitosan-Ag nanoparticles. These nanoparticles also decreased the total count of fecal coliform, fecal streptococci, *P. aeruginosa*, and *S. aureus*, significantly (Hamad 2019). TiO2-NPs generate highly reactive hydroxyl (OH), superoxide (O) and peroxyl radicals (O2). Free radicals alter the structure of cell membranes, causing apoptosis and sterilization.

The nanodevices are useful to enhance the water quality in fisheries, reducing the water exchange rate, increasing rate of survival and yield of fishes. Aquaculture uses activated alumina or carbon nano-materials with zeolite and compounds having iron to hold aerobic and anaerobic biofilm to remove contaminants (nitrites, ammonia, and nitrate). Similarly, ultra-fine nanoscale iron powder can effectively clean up contaminants like polychlorinated biphenyls, trichloroethane, carbon tetrachloride, and dioxins paving the way for nano-aquaculture.

ii. Biofouling Control

Bio-fouling (buildup of microorganisms, algae, and plants on moist surfaces) is additional problem faced by fisheries. Bio-fouling reduces water flow, oxygen ranks, and food bioavailability, while increasing weight, deterioration and cage deformation. Nanotechnology can help enhance disease management, feed formulation, and biofouling control in aquaculture and shrimp cultivation. Invertebrates (mussels and barnacles) and algae (diatoms and seaweeds) can be monitored by covering or painting nanostructures with metal oxide nanoparticles such as CuO, ZnO, and SiO2. This can be attained by improving antifouling control and developing an effective antifouling surface.

Aquaculture antimicrobial compounds and new packaging materials for marine items could all benefit from this antifouling. Some nanomaterials (nano-ZnO and nano-CuO) can help remove fouling organisms from fish aquaculture. They act as a barrier to fouling agents due to their great surface area. CuO nanoparticles have been reported to show significant results in controlling bio-fouling. Lanthanide oxide nanoparticles can absorb phosphate from the surrounding water, reducing algal and other microbial growth. Nanotechnologies make NanoCheck, a pool and fishpond cleaner. It works by absorbing phosphates from water and preventing algae growth with 40 nm lanthanum-based particles. Moreover, nanoscale weedicide and soil-wetting agent delivery may help control aquatic weeds in large water bodies and reduce stress from climate change and pollution (Ashraf et al. 2011).

iii. Removal of Heavy Metals from the Water

Ligand-centered nano-coating can be utilized to remove heavy metals because it can be restored by handling the bi-functional self-assembling ligand with the nano-coating medium that was used before. This process can be done in the same place where the ligand was used (Farmen 2009). Nanoparticles of metal oxides, such as nanosized FeO, AlO2, MnO, CeO2, MgO and TiO, having increased surface area and affinity for metal adsorption are currently preferred nanotechnology for wastewater treatment.

For testing how well they can remove metals under different conditions, mathematical models and analytical techniques like XAS and NMM have become very important for developing new technologies and better applications for making metal oxide nanoparticles. For heavy metal removal, ligand-based nanocoating can be used due to its high absorption potential and low cost. Using crystal clear technology for water purification, several metal layers are bonded to one substrate (Farmen 2009). The high reactivity and large surface area of nanomaterials make them ideal for heavy metal removal from water and waste. Heavy metal adsorption is specific to metal oxide nanoparticles such as nanosized FeO, AlO2, MnO, CeO2, MgO and TiO with high surface area and affinity for aqueous systems.

A detailed study of the impact of humic and fulvic acids on heavy metal removal by nanotechnology from aqueous solutions such as photocatalytic, carbon-based, and iron-based nanomaterials was published by Tang et al. (2014). This study also looked into how humic and fulvic acids interact with each other and the environment. Chitosan nanoparticles are used as ligands and absorbents in the process of heavy metals removal. Recent research has focused on removing heavy metals from clays like montmorillonite, bentonite, and kaolinite using chitosan nanoparticles due to clays' inherent ability to remove heavy metals. For the removal of heavy metals from aquatic environment, chitosan-magnetite nano-composites and nano chitosan-clay composites were also used (Fang et al. 2017).

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